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Preparation and photochemistry of *o*-aminocinnamates

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Abstract

A series of 2-aminocinnamic acid esters and amides was prepared and the photochemistry of these compounds was studied. The compounds undergo *trans–cis* photoisomerization followed by cyclization with expulsion of alcohol or amine. The *o*-amino compounds generally have longer wavelength absorption than the corresponding *o*-hydroxycinnamates, and alkyl substitution on the amino group generally shifts the absorption to longer wavelength. On the other hand, substitution on the amino group reduces the cyclization rate constants (k_c), as compared to the unsubstituted parent. A solvent effect on lactamization was found for these compounds, as increasing Tris buffer content accelerates the cyclization process. These compounds are suitable for use as protecting groups for alcohols and amines and they also serve as models for new photo-labile serine protease inhibitors. A biotinylated derivative has been prepared which extends their use as photo-cleavable linkers for proteins and avidin via the biotin–avidin complex. This permits binding and photorelease of thrombin and other serine protease enzymes to avidin affinity columns.
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1. Introduction

The use of photo-cleavable groups has seen a marked increase in recent years. Nucleophilic functional groups have, for example, been protected by photo-removable groups in applications in peptide and nucleic acid syntheses. Photo-cleavable groups such as 2-nitrobenzyl [1,2], substituted benzyloxycarbonyl [3,4], phenacyl, and the 3,5-dimethoxybenzoinyl groups[5] have found widespread use in protecting both carboxylic acids, alcohols and amines in organic synthesis. A more recent addition to the class of compounds used as photo-removable groups for alcohols and amines are the 2-hydroxy-cinnamates. The stable *trans*-ester isomerizes to the *cis* form after absorbing a photon, which subsequently lactonizes (1 → 2 in Scheme 1a). The result of this process is the generation of the alcohol ROH. Based on this light-induced reaction, some phenolic esters of 2-hydroxycinnamic acids have been designed as photo-activatable inverse inhibitors for a series of serine proteases, such as trypsin, thrombin, Factor Xa [6], and chymotrypsin [7]. Several examples relating to the therapeutic and diagnostic applications of acyl-serine proteases have been reported [8–10]. This system also found recent use as a controlled-release of pro-fragrances, see Scheme 1b [11]. A recent example of 2-hydroxycinnamates illustrated in Scheme 1c is their use in 2-photon photo-release applications when coupled to the well-known *o*-nitrobenzyl protecting group [12].

o-Aminocinnamates also undergo phototransformations in the same manner as the *o*-hydroxy compounds and a few reports have shown their utility as photo-cleavable groups. For example, both the solid phase-bound *o*-hydroxycinnamate and *o*-aminocinnamate could be photolytically cleaved from beads, Scheme 1d [13]. Also, (*E*)-3-(2-amino-4, 5-dimethoxyphenyl)-2-methyl-2-propionic acid phenethylamide can be photolyzed to give 6,7-dimethoxy-3-methylcarbostyril and 2-phenethylamine [14]. The aminocinnamate may indeed be a more versatile functionality than the corresponding hydroxycinnamate since the amino group

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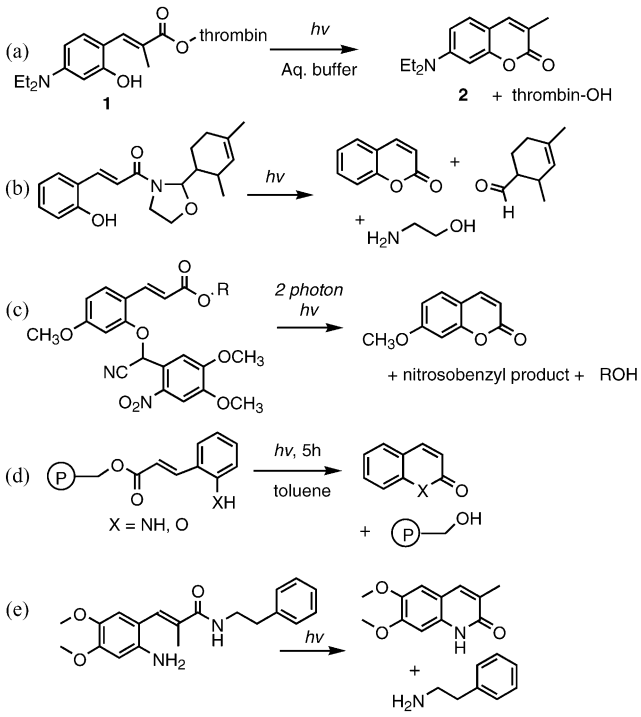


Scheme 1.

provides an additional site for substitution compared to the hydroxyl. Thus, biologically interesting functionality could, in theory, be coupled to this photoactive moiety at the *o*-amino group for various applications. As a thorough characterization of this system has not been previously pursued, we report here on the preparation and the photochemical properties of several *o*-aminocinnamates.

2. Materials and methods

2.1. General

All chemicals were purchased from Sigma–Aldrich except solvents. ^{1}H and ^{13}C NMR spectra were obtained on one of the following NMR spectrometers: General Electric QE-300 MHz, Varian Unity INOVA 400 MHz, Bruker AVANCE 300 MHz, and AVANCE 400 MHz. Infra-red spectrometry was performed with Bomem MB-100 infra-red spectrometer. UV–vis spectra were obtained from a Hewlett Packard 8452A diode array UV–vis spectrometer. Fluorescence spectra were obtained with ISI PC1 photon counting Fluorimeter (ISI Inc., Urbana-Champaign, IL) with a Xenon arc lamp as illumination source (ILC Technology, Sunnyvale, VA). Fischer-brand fluorescence cells (1 cm × 1 cm × 4 cm) were used.

2.2. Synthesis of ethyl 2-aminocinnamate esters [15]

Detailed synthetic procedures are given here for **9**–**12**, **13a**, **14a** and **19**. All compounds prepared (**3**–**21**, **a**–**g**) were fully characterized by ^{1}H NMR, ^{13}C NMR, UV, and high resolution mass spectrometry (HRMS) and/or elemental analysis. Illustrative data is presented for selected compounds here and that for all compounds reported is available elsewhere [15c,15d].

2.2.1. (4-Methyl-3-nitrophenyl)-carbamic acid tert-butyl ester, *9*

Di-*tert*-butyl dicarbonate (34.4 g, 158 mmol) was added to a stirred solution of 4-methyl-3-nitroaniline (20.0 g, 131 mmol) and DMAP (8.0 g, 66 mmol) in CH_3CN (150 mL) over 15 min and the mixture was warmed at 80 °C until dissolution was completed. After stirring/refluxing for 10 h, solvents were removed under reduced pressure, and the residue was partitioned between EtOAc and 10% citric acid. The organic layer was washed with water and brine, and dried over Na_2SO_4. The filtered solution was concentrated under reduced pressure and the residue was flash-chromatographed over silica gel. Elution with 4:1 hexane–ethyl acetate gave product. Yield: 28.23 g (85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (d, 1H, *J* 2.4 Hz), 7.51–7.48 (m, 1H), 7.27–7.22 (m, 1H), 6.51 (s, 1H), 2.53 (s, 3H), 1.53 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.5, 149.0, 137.4, 132.5, 127.4, 122.5, 114.0, 81.2, 28.3, 19.9. FAB-HRMS calcd for $C_{12}H_{16}N_2O_4^+$, 252.1110; found 252.1125.

2.2.2. (4-Formyl-3-nitrophenyl)carbamic acid tert-butyl ester, *10*

A solution of compound 9 (12.0 g, 47.6 mmol), *N*-dimethoxymethyl-*N*,*N*-dimethylamine (15.8 mL, 119 mmol), and pyrrolidine (4.0 mL, 47.6 mmol) in DMF (15 mL) was heated at 140 °C for 10 h under argon. Then the solvent was removed under reduced pressure and a second portion of *N*-dimethoxymethyl-*N*,*N*-dimethylamine (7.9 mL, 59.5 mmol) and pyrrolidine (2.0 mL, 23.8 mmol) was added. The reaction was kept at 140 °C under argon protection till all starting material was consumed. The solution was concentrated under reduced pressure. The dark red residue was redissolved in 50 mL 50% THF/H_2O, and a $NaIO_4$ solution (30.52 g, 142.7 mmol in 50 mL 50% THF/H_2O) was added and stirred at room temperature for 1.5 h. Then the insoluble $NaIO_4$ and $NaIO_3$ were removed by filtration and were washed with EtOAc. The combined organic layer was washed with saturated $NaHCO_3$ solution (2 mL), dried over Na_2SO_4, and the solvent was removed. Flash chromatography over silica gel eluted by 4:1 hexane–ethyl acetate gave product. Yield: 5.0 g (43%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.34 (d, 1H, *J* 5.7 Hz), 8.26 (d, 1H, *J* 2.1 Hz), 7.94 (d, 1H, *J* 8.4 Hz), 7.64–7.61 (m, 1H), 6.96 (s, 1H), 1.55 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 186.7, 151.1, 144.2, 130.9, 124.2, 121.6, 112.7, 87.3, 82.4, 28.0. FAB-HRMS calcd for $C_{12}H_{15}N_2O_5^+$, 267.0981; found 267.0979.

2.2.3. 3-(4-tert-Butoxycarbonylamino-2-nitrophenyl)-2-methylacrylic acid ethyl ester, *11*

Compound **10** (3.0 g, 11.3 mmol) and (carbethoxyethylidene)triphenyl-phosphorane (5.3 g, 14.6 mmol) were

dissolved in 30 mL benzene and stirred overnight at R.T. under subdued light. The solvent was removed under reduced pressure. Flash chromatography over silica gel with elution of 4:1 hexane–ethyl acetate gave product. Yield: 3.73 g (94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (d, 1H, *J* 2.1 Hz), 7.82 (s, 1H), 7.68–7.65 (m, 1H), 7.13 (d, 1H, *J* 8.7 Hz), 6.91 (s, 1H), 4.25 (q, 2H, *J* 7.1 Hz), 1.91 (d, 3H, *J* 1.35 Hz), 1.54 (s, 9H), 1.32 (t, 3H, *J* 6.96 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.4, 152.9, 148.5, 140.1, 135.7, 130.2, 129.0, 125.7, 122.9, 114.6, 81.9, 61.5, 28.6, 14.6, 14.4. FAB-HRMS calcd for $C_{17}H_{23}N_2O_6^+$, 351.1556; found 351.1560.

2.2.4. 3-(4-Amino-2-nitrophenyl)-2-methylacrylic acid ethyl ester, **12**

A solution of 7 mL TFA in 10 mL CH_2Cl_2 was added to compound 11 (3.2 g, 9.1 mmol) cooled by ice bath, and the solution was stirred for 1.5 h, followed by removal of the solvent under vacuum. The crude product was recrystallized in absolute ethanol. Yield: 2.1 g (91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79 (t, 1H, *J* 0.6 Hz), 7.35 (d, 1H, *J* 2.5 Hz), 7.14 (d, 1H, *J* 8.3 Hz), 6.87 (m, 1H), 4.23 (q, 2H, *J* 7.1 Hz), 3.59 (br, s, 2H), 1.93 (s, 3H), 1.32 (t, 3H, *J* 7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.1, 148.8, 147.3, 135.6, 132.2, 128.7, 120.6, 118.9, 109.9, 60.9, 14.2, 14.0. FAB-HRMS calcd for $C_{12}H_{15}N_2O_4^+$, 251.1032; found 251.1038.

2.2.5. 3-(4-Dimethylamino-2-nitrophenyl)-2-methylacrylic acid ethyl ester, **13a**

To a mixture of 12 (400 mg, 1.6 mmol) and K_2CO_3 (80 mg, 0.6 mmol), trimethylphosphate (2.2 mL, 19.1 mmol) was added and stirring was continued at 140 °C for 10 h. Flash chromatograph over silica gel with elution of 4:1 hexane–ethyl acetate gave product. Yield 420 mg (95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (s, 1H), 7.32 (d, 1H, *J* 2.7 Hz), 7.18 (d, 1H, *J* 8.68 Hz), 6.90–6.87 (m, 1H), 4.24 (q, 2H, *J* 7.2 Hz), 3.06 (s, 6H), 1.96 (d, 3H, *J* 1.4 Hz), 1.32 (t, 3H, *J* 7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.2, 150.1, 149.1, 135.7, 132.0, 128.1, 117.9, 116.7, 107.9, 60.8, 40.1, 14.3, 14.1. FAB-HRMS calcd for $C_{14}H_{18}N_2O_4^+$, 278.1267; found 278.1266.

2.2.6. 3-(2-Amino-4-N,N-dimethylaminophenyl)-2-methylacrylic acid ethyl ester, **14a**

Compound **13a** (800 mg, 2.9 mmol) and $SnCl_2{\cdot}2H_2O$ (2.7 g, 14 mmol) were suspended in 15 mL absolute ethanol and kept at 70–80 °C for 30 min. The reaction was cooled down and poured into ice–water. The pH of mixture was adjusted to 7–8 by adding 5% $NaHCO_3$. The mixture was extracted with CH_2Cl_2. The organic phase was washed with brine and dried with Na_2SO_4. The solvent was removed under reduced pressure. Flash chromatography over silica gel with elution of 4:1 hexane–ethyl acetate gave product. Yield: 550 mg (77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (s, 1H), 7.09 (d, 1H, *J* 8.6 Hz), 6.23–6.20 (m, 1H), 6.03 (d, 1H, *J* 2.5 Hz), 4.22 (q, 2H, *J* 7.1 Hz), 3.75 (s, 2H), 2.95 (s, 6H), 2.06 (d, 3H, *J* 1.4 Hz) 1.32 (t, 3H, *J* 7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.9, 151.5, 146.3, 134.6, 130.7, 125.0, 110.4, 103.0, 98.3, 60.3, 39.8, 14.1. FAB-HRMS calcd for $C_{14}H_{20}N_2O_2^+$, 248.1525; found 248.1533.

2.2.7. 3-(2-Amino-4-N,N-dimethylaminophenyl)-2-methylacrylic acid, **19**

The 3-(2-amino-4-dimethylaminophenyl)-2-methylacrylic acid ethyl ester, **14a** (900 mg, 3.6 mmol) was dissolved in 4 mL ethanol and NaOH solution (434 mg, 11 mmol in 5 mL water) was added, and stirred at 80–90 °C for 6 h. After removal of the ethanol, the remaining solution was washed with diethyl ether, and then acidified to pH 6–7 with 6N HCl. The crude product was extracted into CH_2Cl_2, which was dried over Na_2SO_4 and evaporated to give a yellow solid. The crude acid was recrystallized from ethyl acetate to give a crystal. Yield: 80%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.15 (d, 1H, *J* 8.7 Hz), 6.21 (m, 1H), 6.02 (d, 1H, *J* 2.4 Hz), 2.96 (s, 6H), 2.10 (d, 3H, *J* 1.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.2, 152.1, 147.5, 139.2, 136.8, 131.1, 128.7, 127.4, 127.2, 124.0, 109.7, 101.6, 94.7, 48.3, 40.2, 14.3. FAB-HRMS calcd for $C_{12}H_{16}N_2O_2^+$, 220.1212; found 220.1208.

2.3. Preparative formation of carbostyrils

The carbostyrils (**15**–**18**) were prepared from photolysis of the corresponding ethyl cinnamate esters (**5**–**7**, **14**) respectively. A general procedure is given here. All compounds prepared were fully characterized. Illustrative spectroscopic data for **18a** is reported here and that for all compounds reported is available elsewhere [15c,15d].

2.3.1. General procedure

About 30–50 mg of the substituted 3-(2-aminophenyl)-2-methylacrylic acid ethyl ester was dissolved in 50 or 100 mL absolute ethanol. The solution was stirred and purged with argon for 10 min. A Kimberly Kimax round bottom flask was used as container. The light source was a Hanovia low pressure UV lamp (450 W) filtered by Pyrex sleeve. The distance between the light source and the solution is 20 cm for **5**–**7** and 8 cm for **14a**–**c**. TLC was used to monitor the progress of photolysis till all starting material was consumed. The quinolin-2-one photoproduct was purified with flash chromatography (SiO_2, EtOAc/hexane).

2.3.2. 7-Dimethylamino-3-methyl-1H-quinolin-2-one, **18a**

Yield: 89%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.09 (s, 1H), 7.48 (s, 1H), 7.30 (d, 1H, *J* 8.8 Hz), 6.64–6.61 (m, 1H), 6.35 (d, 1H, *J* 2.4 Hz), 3.04 (s, 6H), 2.20 (s, 3H). ^{13}C NMR (75 MHz, CD_3COOD): δ 167.1, 153.0, 141.0, 140.0, 129.7, 124.2, 114.3, 112.4, 98.0, 41.4, 16.9. FAB-HRMS calcd for $C_{12}H_{15}N_2O^+$, 203.1184; found 203.1204.

2.4. Preparation of an aminocinnamate photo-cleavable linker, **24**

2.4.1. (E)-Ethyl 3-(4-(dimethylamino)-2-((ethoxycarbonyl)methylamino)phenyl)-2-methylacrylate, **22**

To **14a** (50 mg, 0.201 mmol) in 4 mL DMF was added 26 μL ethyl iodoacetate. Stirring at room temperature as continued for 60 h. Flash chromatography over silica gel with elution of 3:1 hexane–ethyl acetate gave product. Yield 48 mg (75%) ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.63 (s, 1H), 7.07 (d, 1H, *J* 6.8 Hz), 6.16 (dd, 1H, *J* 2.4 Hz, *J* 8.6 Hz), 5.82 (d, 1H, *J* 2.3 Hz), 4.36 (s, 1H), 4.22–4.29 (m, 4H), 3.89 (s, 2H), 2.97 (s, 6H), 2.04 (d, 3H, *J* 1.2 Hz), 1.28–1.36 (m, 6H). 13NMR (75 MHz, CDCl3): δ (ppm) 170.9, 168.9, 151.8, 146.2, 134.5, 131.0, 126.6, 110.6, 102.1, 94.5, 61.3, 60.52, 46.0, 40.3, 14.4, 14.2. FAB-HRMS calcd. $C_{18}H_{26}N_2O_4^+$ for 334.1893, found 334.1882.

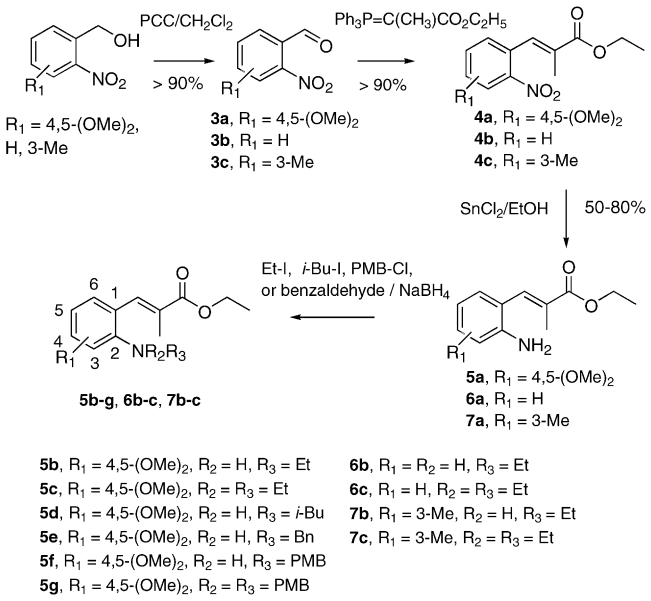
2.4.2. 2-(5-(Dimethylamino)-2-((E)-2-(ethoxycarbonyl)prop-1-enyl)phenylamino)acetic acid, **23**

The diester **22** (250 mg, 0.748 mmol) was dissolved in 5 mL EtOH and NaOH solution (30 mg, 0.748 mmol in 5 mL water) was added and the solution was stirred at room temperature for 8 h. Ethanol was removed by vacuum and the resulting solution was acidified to pH 6 with 6N HCl and was extract into EtOAc. Solvent was removed by vacuum to get 200 mg yellow solid which was used directly for the next step (87%). This product is very unstable and should be kept under Argon in the refrigerator. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.62 (s, 1H), 7.05 (d, 1H, *J* 5.4 Hz) 6.63 (s, 2H), 6.20 (dd, 1H, *J* 2.3 Hz, *J* 8.6 Hz), 5.88 (d, 1H, *J* 2.2 Hz), 4.22 (q, 2H, *J* 7.1 Hz), 3.99 (s, 2H), 2.96 (s, 6H), 2.04 (d, 3H, *J* 1.2 Hz), 1.31 (t, 3H, *J* 7.1 Hz), ^{13}C NMR (75 MHz, CDCl3): δ (ppm) 169.1, 151.7, 146.2, 134.6, 131.0, 126.8, 111.0, 102.7, 95.1, 60.7, 45.9, 40.5, 40.3, 14.4, 14.3. FAB-HRMS calcd. For $C_{16}H_{22}N_2NaO_4^+$, 329.1477; found 329.1489.

The conversion of **23**–**24** involves standard amide and ester coupling procedures. All intermediates were fully characterized by ^{1}H NMR, ^{13}C NMR, UV, and high resolution mass spectrometry (HRMS) and/or elemental analysis. Spectroscopic data for all compounds reported is available elsewhere [15d].

2.5. Photochemical studies

A 1000 W ozone-free Hg/Xe lamp (Oriel 6295) mounted in a lamp housing (Oriel 66021) was used for the photochemical bench. An Oriel 68820 power supply was used for lamp ignition and operation. The stable operating current was 29.5 A at 32 V. Along the light pathway are properly aligned an IR filter (cooled by running water), entrance-slit focusing optics, monochromator, exit-slit focusing optics, and sample chamber.



Scheme 2.

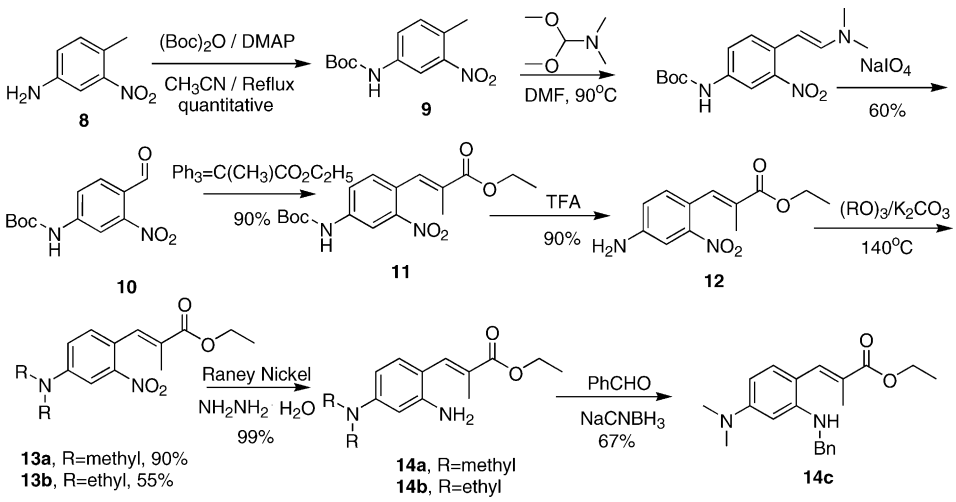
Use of the photochemical bench for general photochemical experiments is as follows. A known concentration of substrate (**5**–**7**) in a controlled solvent composition was added to a quartz cuvette and fixed in the photolysis chamber. The irradiation at a selected wavelength was applied for a defined amount of time and the following dark reaction was monitored by either HP 8542A diode UV spectrophotometer for a full spectrum, or the SPECTRAmax® PLUS microplate spectrophotometer at single wavelength (Molecular Devices, Sunnyvale, CA). For NMR-monitoring deuterated solvent was utilized and the solution was contained in an NMR tube and was fully exposed to incident light.

For lactamization kinetic measurement, a solution in ethanol was subject to irradiation at a single wavelength (366 or 342 nm) for 5 min while stirred. Then an aliquot of ethanolic solution was withdrawn and add to a known volume of Tris buffer (50 mM, with 150 mM NaCl, pH 7.4, same in the following context) in a quartz UV cell. After vortexing for 10–15 s the cell was placed immediately in a UV spectrophotometer. The wavelength at the absorption maximum of the corresponding carbostyril was recorded over 0.5–4 h at 26.5 ± 0.1 °C, depending on the rate of lactamization. The kinetic data thus recorded was subject to regression (exponential increase to maximum, 3-parameters) with SigmaPlot 5.0 to deduce the rate constant.

3. Results and discussion

3.1. Synthesis and spectral characterization

Compounds **5**–**7** were synthesized as shown in Scheme 2. The *o*-nitroaldehydes were prepared in excellent yield from the corresponding benzyl alcohols by PCC oxidation and the

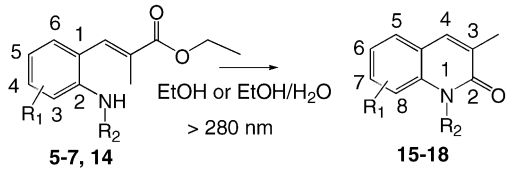


Scheme 3.

subsequent Wittig reaction is quantitative. Reduction of the nitro group to amine was achieved by the use of tin(II) chloride. The *ortho*-amino group of the cinnamates are rather inert but could still be alkylated in one of the following ways: (1) Nucleophilic alkylation with excess ethyl iodide (or *iso*-butyliodide) in DMF in the presence of silver nitrate followed by chromatographic isolation of desired products. (2) Reductive amination with benzaldehyde and sodium borohydride. This method only worked well for aromatic aldehydes. (3) Reaction with benzylic halide such as *p*-methoxybenzyl chloride gave both mono- and di-PMB amines in good yield.

The preparation of 4-amino substituted cinnamates is outlined in Scheme 3. The aldehyde, **10**, is not commercially available and the chemistry described provides this key intermediate in good overall yield from the available starting material. Reductive amination of **14a** with benzaldehyde–cyanoborohydride gave the benzyl derivative, **14c**.

The photolysis product of compounds **5**–**7** and **14** are shown in Scheme 4. Yields are good to excellent for the photo-conversion.



15a, R_1 = 4,5-$(OMe)_2$, R_2 = H
15b, R_1 = 4,5-$(OMe)_2$, R_2 = Et
15d, R_1 = 4,5-$(OMe)_2$, R_2 = *i*-Bu
15e, R_1 = 4,5-$(OMe)_2$, R_2 = Bn
16a, $R_1 = R_2$ = H
16b, R_1 = H, R_2 = Et
17a, R_1 = 3-Me, R_2 = H
17b, R_1 = 3-Me, R_2 = Et
18a, R_1 = 7-Me_2N, R_2 = H
18b, R_1 = 7-Me_2N, R_2 = Bn
18c, R_1 = 7-Et_2N, R_2 = H

Scheme 4.

3.2. UV–vis and fluorescence spectra of 5–7, 14–18

The UV spectra of all photo substrates and products were taken in either ethanol or 90% Tris buffer/ethanol (v/v, Table 1). The fluorescence spectra for **15a**, **b**, **d**, **e** and **18a**–**c** were acquired in the same Tris–ethanol buffer. Two excitation wavelengths, 342 and 366 nm were used for the fluorescence experiments (Table 2). For the 6,7-dimethoxyquinolinones (**15a**, **b**, **d**, **e**), the λ_{em} is close to λ_{ex}.

The absorption maxima for compounds **5**–**7** is presented in Table 3. It is of interest to note that mono-alkyl substitution on the *o*-amino group red-shifts the absorption of compounds **5** and **6** by some 15–20 nm while *N*,*N*-di-alkyl substrates are all blue-shifted. The red-shift of one alkyl substituent may be attributed to the hyperconjugation effect between the alkyl group and the aniline in the excited state [16], as the C–H σ bond may overlap with the highly polar planar delocalized ^{1}S state. Due to steric interactions, the dialkyl-substituted anilines (**5c**, **6c**) and **7b** cannot assume a planar conformation, resulting in shorter wavelength λ_{max}. The carbostyrils **15**–**17** have shorter absorption maximum wavelength than their corresponding precursors (**5**–**7**), yet the extinction coefficients are almost doubled.

3.3. Tautomers of the photolytic products

Carbostyril compounds undergo tautomerization between the lactam (quinolin-2-one, Q) and the lactim (2-hydroxy quinoline, HQ) forms in solution [17]. The thermodynamic equilibrium is highly solvent dependent. Polar solvents drive the equilibrium to the side of Q. In aqueous medium the Q form is favored by 5 kcal/mol, because the polar and hydrogen bonding apparently stabilize this form. Both forms of quinilin-2(1*H*)-one have been shown to exist in the gas phase [18]. In the solid state, quinolinone has been assigned by X-ray crystallography to be in the Q form [19].

Proton and ^{13}C NMR [20,21], IR, and UV spectra all suggest that the photo-products generated here all exist in the

Table 1
UV–vis absorption of compounds **5**–**7**, **14**–**18**

	$\lambda_{max}/\varepsilon$ (ethanol)	$\lambda_{max}/\varepsilon$ (Tris–ethanol)[a]		$\lambda_{max}/\varepsilon$ (ethanol)	$\lambda_{max}/\varepsilon$ (Tris–ethanol)[a]
5a	356/6.90 × 10^3	342/6.67 × 10^3	**15a**	342/1.30 × 10^4	338/1.37 × 10^4
5b	370/6.46 × 10^3	352/5.50 × 10^3	**15b**	342/1.12 × 10^4	338/1.14 × 10^4
5d	374/7.07 × 10^3	356/5.84 × 10^3	**15d**	344/1.05 × 10^4	338/1.08 × 10^4
5e	372/6.76 × 10^3	356/5.02 × 10^3	**15e**	344/1.15 × 10^4	340/1.22 × 10^4
6a	338/4.59 × 10^3	322/4.15 × 10^3	**16a**	324/7.27 × 10^3	322/7.75 × 10^3
6b	354/3.96 × 10^3	334/3.34 × 10^3	**16b**	326/6.41 × 10^3	322/6.54 × 10^3
7a	338/4.64 × 10^3	322/3.58 × 10^3	**17a**	328/6.42 × 10^3	324/6.34 × 10^3
7b	328/3.90 × 10^3	314–322/3.35 × 10^3	**17b**	328/5.28 × 10^3	326/5.30 × 10^3
14a	362/6.55 × 10^3	352/7.8 × 10^3	**18a**	356/6.50 × 10^3	352/6.36 × 10^3
14b	366/6.13 × 10^3	366/7.9 × 10^3	**18b**	360/4.95 × 10^3	356/5.87 × 10^3
14c	374/1.01 × 10^4	388/6.46 × 10^3	**18c**	364/8.59 × 10^3	364/7.42 × 10^3

[a] The solvent mixture is Tris buffer (pH 7.4)/ethanol 90% (v/v).

Table 2
Emission Maximum of **15a**, **b**, **d**, **e**, **18a**–**c**

	Solvent	Excitation, 366 nm	Excitation, 342 nm
15a	[a]	394	388
	[b]	392	392
15b	[a]	390	388
	[b]	391	391
15d	[a]	391	395
15e	[a]	392	392
18a	[a]	447	446
18b	[a]	442	442
18c	[a]	446	445

In this solvent, compound **15a** has λ_{max} 342 nm (ε 1.58 × 10^4 cm^{-1} M^{-1}), and compound **15b** has λ_{max} 342 nm (ε 1.23 × 10^4 cm^{-1} M^{-1}).
[a] Tris buffer (pH 7.4)/ethanol 90% (v/v).
[b] Dichloromethane.

Q form in respective media. The photo-products from the *N*-ethyl substrates have similar spectral characteristics to the unsubstituted Q form quinolinones, supporting the structural assignment. Indeed, the structure of photo-product **15b** has been unambiguously assigned by NOE ^{1}H NMR as being the *N*-ethyl-quinolin-2-one.

3.4. Kinetics of photo-conversions

The photochemical conversion of **5a** to **15a** and **5e** to **15e** was monitored by ^{1}H NMR. A series of NMR spectra of **5a** in 50% D_2O/CD_3OD taken after photolysis (366 nm) showed the clean and near quantitative conversion to **15a**, with no observable intermediates. Analysis of the photolysis of **5e** in 62% D_2O/CD_3OD however, showed an intermediate, after each round of irradiation, which lived long enough to be recorded by NMR. After several hours in the dark, the intermediate disappeared and product formed. By analogy to the photolysis of compound 1 in THF at low temperature [19], we suggest that the intermediate is the *cis*-isomer of **5e**. Apparently the *N*-substituted 2-aminocinnamate cyclizes more slowly than the unsubstituted one.

Table 3
Influence of the *N*-alkyl substituents on the maximum absorption wavelength of **5**–**7** in ethanol

Ar-NH_2	Ar-NHR	Ar-NR_2
5a/356	**5b**/370	**5c**/334
	5d/374	
	5e/372	
	5f/372	**5g**/332
6a/338	**6b**/354	**6c**/332
7a/338	**7b**/328	**7c**/318–334

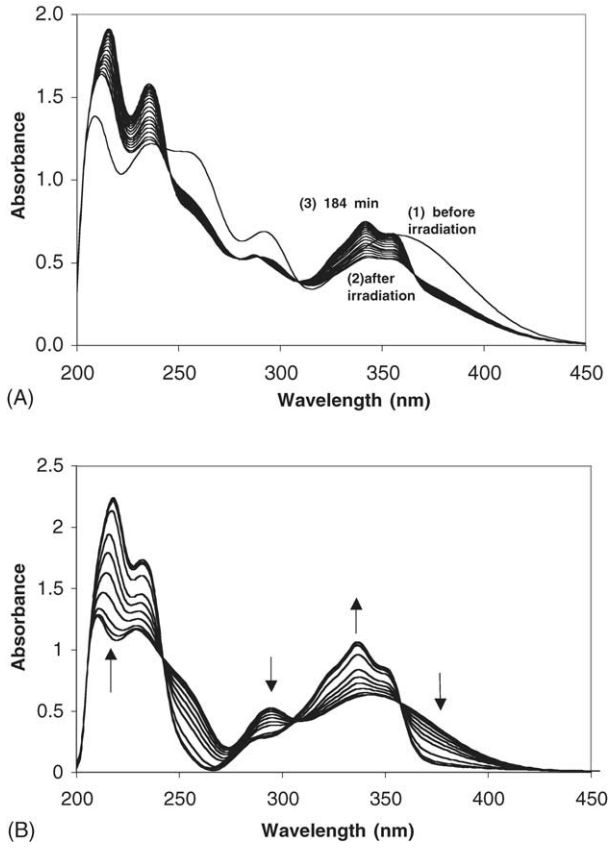


Fig. 1. (A) Photolysis and dark cyclization of **5a** in ethanol. Isosebestic points at 246, 290, 310, 366 nm. The spectra were taken at (1) before irradiation; (2) after 5 min irradiation at 366 nm (t = 0); and (3) followed by darkness at R.T. for 0, 1, 2, 4, 6, 8, 10, 12, 16, 20, 25, 30, 35.2, 40, 45, 52, 60, 70, 90, 120, 150, 184 min, respectively. (B) Photo-conversion of **5a**–**15a** in Tris buffer (pH 7.4, with 10% ethanol). Isosebestic points at 242, 306, 358 nm.

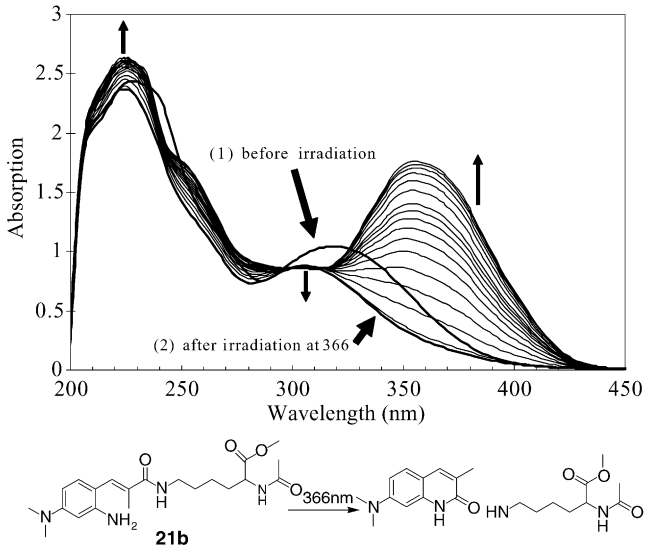


Fig. 2. Photoconversion of **21b** in pH 4.1 buffer. Spectra were taken at (1) before irradiation; (2) after 6 min irradiation at 366 nm (t = 0); and (3) followed by darkness at R.T. for 0, 2, 12, 22, 22, 32, 42, 52, 62, 72, 82, 92, 117, 147, 177, 207, 237, 267 min. Isosebestic points at 240, 298, 314 nm.

A UV–vis study provided additional evidence for the *trans*-to-*cis*-to-lactam mechanism. Photolysis of compounds **5**–**7** in pure ethanol gave a quick change of UV spectra, followed by a slow conversion to lactam-like spectra. The change of UV spectrum of **5a** in absolute ethanol over a period of 3 h after an initial 5 min irradiation is presented in Fig. 1A.

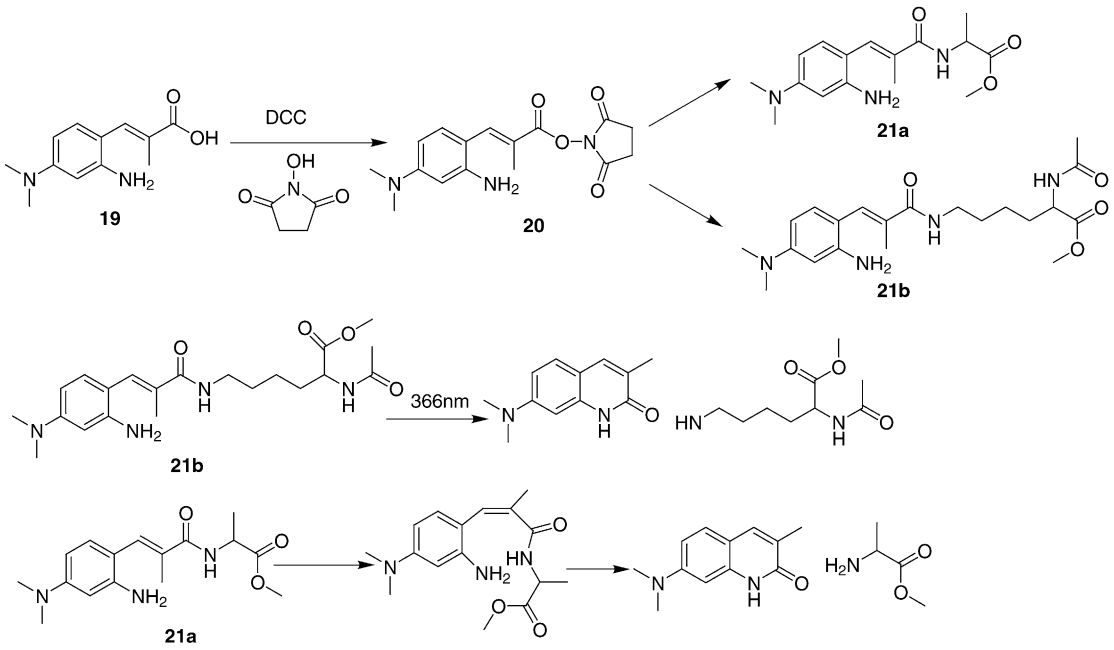
In contrast, photolysis of the same compound in Tris buffer with 10% ethanol gave spectra at various irradiation times with isosbestic points (Fig. 1B for **5a**), suggesting that no significant amount of intermediate accumulated and that the cyclization of *cis*-5–7 to **15**–**17** was fast and clean in this media.

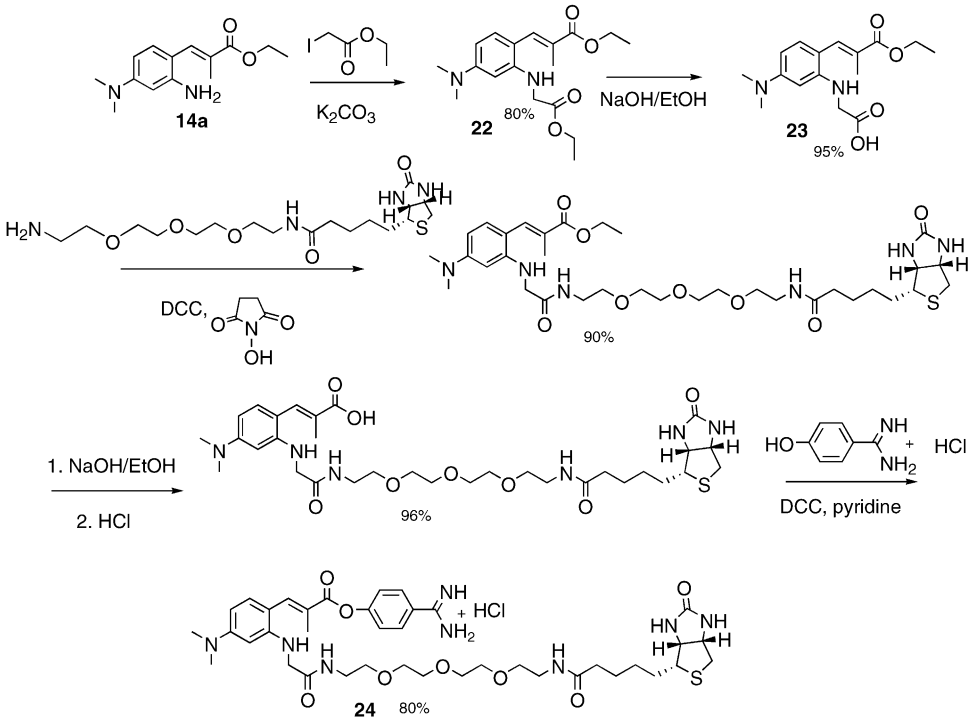
The dark lactamization rate in a series of Tris buffer/ethanol mixtures was then determined by monitoring the UV change of irradiated samples over time. Good first-order kinetics was observed for converting compounds **5a**, **b**, **6a**, **b**, and **7a**, **b** to corresponding carbostyrils, and the rate constant for lactamization (cyclization), k_c, could be obtained by this observation [15c,15d]. The solvent composition effect on lactimization rate is demonstrated for **5a** as follows. As the buffer changes from 90% Tris–10% ethanol to 50:50 to 100% ethanol, the half-life of the *cis*-intermediate changed from 0.62 to 4.5 min to 14.3 min. Alkylation of the anilinic nitrogen also increases the half-life for cyclization. For example, in same 90% Tris buffer *cis*-**5b** lactamizes with a half-life of 2.7 min, compared to 0.62 min for *cis*-**5a** [15c,15d].

We make the further generalizations that there is a nearly linear relationship between log k_c with respect to Tris buffer percentage in the media. Previous work has shown that the k_c for *cis*-1 depends on the Tris buffer content of the media [22]. The same trend was observed for **5a**, **b**, **6a**, **b**, and **7a**, **b**. The cyclization of the unsubstituted substrates (**5a**, **6a**) is four times faster than the corresponding *N*-ethyl compounds (**5b**, **6b**). Compounds **7a** and **7b**, in contrast, have nearly identical cyclization rates, and in low Tris content solvent, **7b** cyclizes even faster than **7a** [15c,15d].

3.5. Amine protection and photo-deprotection

Studies were carried out on one of the *o*-aminocinnamates in order to assess its utility as a photo-cleavable protecting group. Thus, the ester **14a** was hydrolyzed with NaOH/ethanol to give **19**, 80%, and this acid was coupled with *N*-hydroxypyrrolidine-2,5-dione providing **20** in 90% yield in a reaction catalyzed by DCC. The compound **20** proved to be a useful reagent for the preparation of cinnamate modified peptide residues. Substrates **21a** and **b** were prepared directly from **20** in excellent yield.



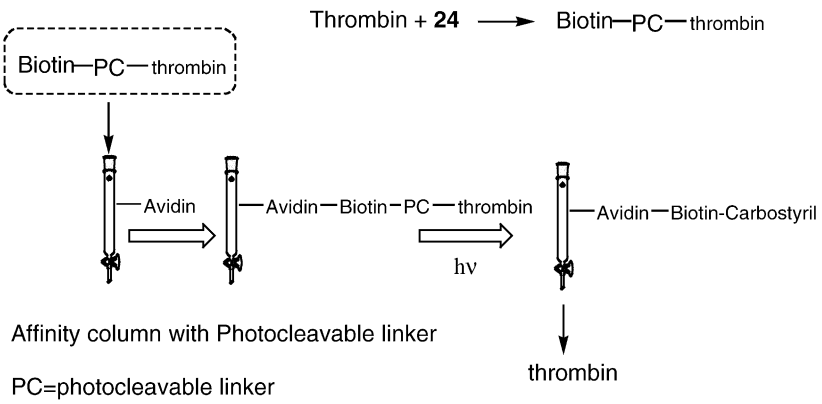


Scheme 5.

Efficient photo-release of peptide from **21a** and **b** (90–100%) occurs after photolysis in pH 4 or 5 buffer while photolysis at pH 7.4 results only in photoisomerization. The change of the UV spectrum of **21b** in pH 4 buffer after an initial 6 min irradiation is presented in Fig. 2. An intermediate, presumably *cis*-**21b**, is observed in this experiment after photolysis. This intermediate converts to lysine and carbostyril with an apparent half-life of about an hour. Under similar conditions of photolysis, no intermediate is observed during photolysis of **14a**, the ethyl ester analog of **21b**. Photo-release of an alcohol is much faster than release of an amine, and consequently no *cis* intermediate is observed during the photolysis of **14a** [15c,15d]. Experiments are underway to provide additional information about factors controlling the rate of photo-release of amines.

3.6. Aminocinnamate photo-cleavable linkers

To illustrate the utility of the *o*-aminocinnamates, **14a** was modified as shown in Scheme 5 to permit its use as a photo-cleavable linker. In this example, biotin was attached at the *o*-amino group and the cinnamate was modified such that it served as a photoreversible inhibitor of serine proteases such as thrombin and Factor Xa. The yields for conversion of **14a**–**24** are good to excellent and multi-milligram quantities of the final protease substrate could be prepared [15d]. While most of the transformations shown in Scheme 5 are unexceptional, the selective hydrolysis of the di-ester **22** is noteworthy. The cinnamate ester is more resistant to hydrolysis than the 2-amino ester because of polar and steric effects.



Scheme 6.

Biotinylated compounds are retained on columns by affinity with immobilized monomeric avidin. Following elution with a regeneration buffer, the biotinylated compounds are released. This method is limited by the difficulty of removing the biotin label from the desired molecule, i.e., to recover the target molecule in an unmodified and biologically functional form [67]. The introduction of photo-cleavable tags (PC tags) solves problems associated with traditional affinity labels by photochemically releasing the modified compound. Once the target compound is bound to the column through avidin–biotin interaction, it can be easily separated from non-biotinylated compounds and released from the column by irradiation. One example of this strategy, which is outlined in Scheme 6, has been demonstrated by Thuring and co-workers [10].

The biotinylated 2-amino cinnamate compound **24** inhibits both thrombin and Factor Xa by acylating the active site serine hydroxyl on the cinnamate. Thrombin is acylated about twice as fast as Factor Xa with this inhibitor but both are rendered completely inactive in about an hour when incubated with **24** under conditions described elsewhere [10,23]. The thrombin-**24** acyl enzyme has a half-life of about 40 h at pH 7.4 and 37 °C in the dark. In fact, most of the *o*-aminocinnamate thrombin derivatives have half-lifes for deacylation of 40–60 h, somewhat shorter than that of the thrombin acyl *o*-hydroxycinnamate 1 (138 h).

A solution of completely inactive thrombin, formed by inhibition with inhibitor **24**, was applied to a monomeric avidin column. The column was eluted with several volumes of pH 7.2 phosphate-buffered saline and no active thrombin was eluted in the fractions collected. The avidin column was then exposed to 366 nm wavelength irradiation for 4–5 min from different sides of the column and the column was subsequently eluted with a (PBS buffer, pH 7.2) solution. Several thrombin-containing fractions were taken from the column and the combined fractions showed activity corresponding to approximately 80% of the thrombin anticipated from photolysis of the acyl enzyme, as measured by standard thrombin assays. This experiment indicates that the *o*-aminocinnamate compounds can be effectively used as photo-cleavable linkers. The biotin modification is but one application of many in which this photolinker provides potential utility. In this instance, the photo-cleavable linker serves to attach the inactive enzyme to a solid support. When irradiated, the fully active enzyme is released from the support.

4. Conclusion

The *o*-aminocinnamate group may prove to be a useful photochemically active functionality for use in various applications. Compounds with this substructure are readily accessible and their photochemistry is well-behaved. They provide an alternate substructure to the *o*-hydroxy analogs that may prove useful in strategies for photo-generation of biologically active agents or in studies of enzymes or proteins with modifiers that are photo-cleavable.

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